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# The effect of cold stress on damping-off of soybean caused by *Pythium sylvaticum*

## Introduction

*Pythium sylvaticum* is an Oomycete that causes damping-off of soybeans in Iowa, particularly when cool and wet weather occurs at planting. Although farmers try to plant soybean when soils are warm (>15°C), it is not uncommon for soil temperatures to drop below 10°C for several days when cold fronts occur soon after planting.

Low temperatures at planting delay germination and thus seedlings may be susceptible to infection for a longer period of time. Also, under high soil moisture conditions greater seed exudation may occur and stimulate nearby pathogens, thus contributing to higher seedling infections. Thus early planted soybean seedlings exposed to adverse conditions are at risk for damping-off that may reduce plant stand or seedling vigor.

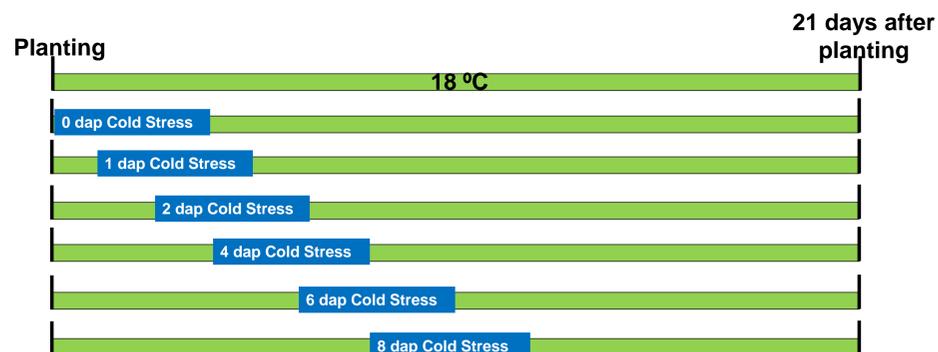
In this experiment we simulated the effect of a period of 96 hours of cold stress (< 10°C) at different times after planting to improve our understanding of the role of cold stress on the occurrence damping-off of soybean caused by *Pythium sylvaticum*.

## Methods

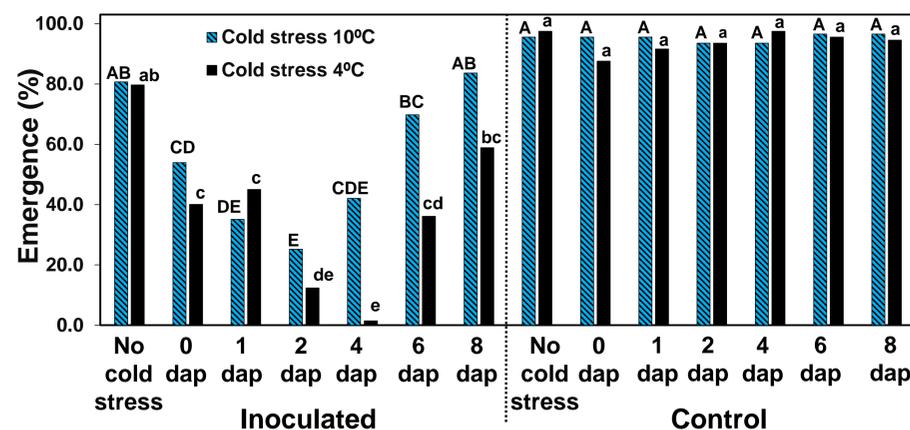
- Soybean seeds IA 2094 were planted into 8 oz cups inoculated with *P. sylvaticum*-infested millet or autoclaved millet seed (Figure 1).
- The cups were placed in growth chamber and subjected to 96 hours of cold stress (4°C or 10°C) at 0, 1, 2, 4, 6 and 8 days after planting (Figure 2). Emergence was evaluated 21 days after planting (Figure 3).
- Seed exudation during imbibition (leakage) was determined with the electrical conductivity test. Seeds were imbibed at 4°C, 10°C and 18°C for 24 hours (Table 1). Also, the seed exudate solution was used for sporangia germination assay.
- Sporangia from *P. sylvaticum* was produced on 3mm culture media disks (diluted V8 juice media), flooded with the exudate solution and incubated at for 3 hours at 24°C. Sporangia was examined at the microscope (X200) and percent of germinated sporangia was determined (Figure 5, Table 2).
- Mycelial growth of *P. sylvaticum* was assessed after 48, 72 and 92 hours in 90-mm-diameter petri dish plates with diluted V8 media (Figure 6).



**Figure 1.** Foam cups are filled with layers of vermiculite, *P. sylvaticum*-infested-millet seed and 10 soybean seeds.



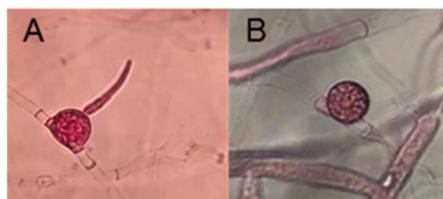
**Figure 2.** Description of cold stress treatments



**Figure 3.** Emergence (%) of soybeans IA 2094 non-inoculated (control) and inoculated with *Pythium sylvaticum* subjected to 96 hours of cold stress (4°C or 10°C) at different days after planting (dap). Different letters indicate significant differences according to Tukey test alpha = 0.05



**Figure 4.** Soybean plants subjected to 96 hours of cold stress (10°C) 2 days after planting. **A**, inoculated with *P. sylvaticum*; **B**, non-inoculated control. Pictures were taken 21 days after planting.



**Figure 5.** *Pythium sylvaticum* sporangia (X200). **A**, germinated sporangium with a germ tube 3 hours after exposure to seed exudate. **B**, non-germinated sporangium 3 hours after exposure to sterile water.

**Table 1.** Solute leakage ( $\mu\text{S}/\text{cm g}$ ) of soybean seed IA 2094 after incubation for 24 hours at different temperatures.

Temperature (°C)	Leakage ( $\mu\text{S}/\text{cm g}$ )
4	65.9 a <sup>2</sup>
10	59.6 b
18	56.9 b
<b>P value</b>	<0.0001

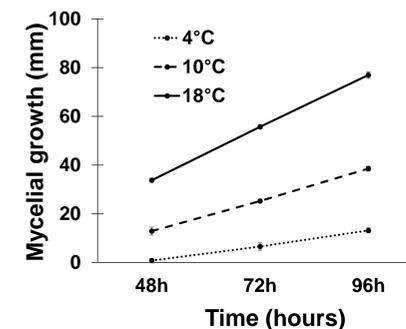
<sup>2</sup>Different letters indicate significant differences according to Tukey test alpha = 0.05.

**Table 2.** Sporangia germination (%) of *Pythium sylvaticum* after exposure to different seed exudates and incubation at 24°C for 2 and 3 hours.

Seed exudate <sup>y</sup>	Germinated sporangia (%) at 24°C	
	2h	3h
Seeds imbibed at 4°C	65.2 a	65.2 a
Seeds imbibed at 10°C	42.9 b	42.9 b
Seeds imbibed at 18°C	48.2 b	48.2 b
Sterile water	0.5 c	0.5 c
<b>P value</b>	<0.0001	

<sup>y</sup>Three seed exudates were prepared by imbibition of soybean seed in sterile water at 4°C, 10°C or 18°C for 24 hours.

<sup>2</sup>Different letters indicate significant differences according to Tukey test alpha = 0.05.



**Figure 6.** Mycelial growth (mm) of *P. sylvaticum* at three different temperatures on diluted V8 media. Error bars indicate standard deviation.

## Conclusions

- Cold stress increased soybean susceptibility to damping-off.
- P. sylvaticum* is able to grow at low temperatures that are suboptimal for soybean growth.
- Greater seed exudation occurred at low temperatures; and the seed exudates stimulated sporangia germination of *P. sylvaticum*.
- These results indicate that periods of cold stress at or soon after planting increase the susceptibility of soybean to damping-off caused by *P. sylvaticum* which could contribute to reduced plant stands and decreased yields.